The impact of oxidative stress on Par2, Ptgs2 genes expression in rat duodenal epithelial cells under conditions of prolonged gastric hypochlorhydria and with administration of multiprobiotic

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ABSTRACT

Development of dysbiosis is a key consequence of long hypoacidity, in which there is colonization of the gastrointestinal tract by pathogenic microflora that forms source of sustainable endogenous infection and leads to inflammation. The prevalence of free-radical processes of an antioxidant defense system is an important pathogenetic link in the origin and development of various disorders. Because reactive oxygen species are important for transcription of Ptgs2 gene, the relationship between oxidative stress, antioxidant element and PAR2-mediated signaling pathways may take place under conditions of dysbiotic changes due to hypochlorhydria. To evaluate the intensity of free radical processes and their impact on Par2, Ptgs2 genes expression in rat duodenal epithelial cells under conditions of prolonged gastric hypochlorhydria and with administration of multi-probiotic. Four experimental groups were created with 8 rats in each. One group was a control, while others were injected with acid suppressant omeprazole or multi-strain probiotic preparation or with both compounds simultaneously for 28 days. Protein oxidative modification products, free SH-groups, metallothioneins content were measured in villus and crypt epithelial cells with standard biochemical assays. Level of Par2, Ptgs2 genes mRNA was determined with semi-quantitative RT-PCR. Prolonged inhibition of acid secretion in stomach was accompanied by significant elevation of free radical processes (intensification of protein oxidative modification, free SH-groups pool reduction, changes of metallothioneins concentration) and elevation of Par2 and Ptgs2 genes expression levels in rat duodenal epithelial cells. Reduction of free radical processes intensity; restoration of redox balance and the normalization of Par2, Ptgs2 gene expression pattern in the duodenum upon the simultaneous administration of the multi-probiotic Symbiter were observed. Par2 over-expression leads to activation Ptgs2 on the background of free radical processes over the antioxidant defense system in the development of inflammation in the duodenum through dysbiotic changes in the conditions of prolonged hypochlorhydria.

INTRODUCTION

The gastric hypochlorhydria has a multifactorial etiology. She accompanies several diseases (atrophic gastritis, gastric cancer, autoimmune disorders) and is the result of pharmaceutical drugs administration - proton pump inhibitors of gastric parietal cells of the stomach, for example, widely representative omeprazole (Shin et al., 2008, Sundstrom et al., 2006, Johnson et al., 2013, Barrett et al., 2006). The development of dysbiosis - a key consequence of long hypoacidity, in which there is colonization of the gastrointestinal tract (GIT) by pathogenic microflora that forms source of sustainable endogenous infection. This leads to inflammation and is an additional factor which besides hypergastrinemy, promotes gastric carcinogenesis and the occurrence of sporadic tumors in other parts of the GIT and its associated organs (Barrett et al., 2006, Thomson et al., 2010).

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Par2 gene (encodes proteinase-activated receptor 2 (PAR2), subgroup of G-protein coupled receptor family) is highly expressed in different pathologies of GIT (Seo et al., 2011; Kamath et al., 2001; Ritchie et al., 2007). PAR2 is activated in response to the partial proteolytic cleavage of the extracellular domain by serine proteases under the influence of p38 MAP (mitogen-activated protein) kinase and by the NFkB signalling pathway, including the potentially divergent roles of the kinases inhibitory kappa B kinases (IKK)-α and –β (Ritchie et al., 2007). Inflammatory cytokines (IL-1α, TNF-α) increase mRNA expression of Par2 in disorders (Seo et al., 2011; Kamath et al., 2001; Ritchie et al., 2007).

Also the increase in this gene expression is decided to consider as a protective mechanism of cells from premature activation of proteolytic enzymes (Gorelick, 2007). Par2 modulates activation of proinflammatory genes and genes involved in carcinogenesis, for example, Ptgs2 (encoding cyclooxygenase-2, COX-2) due to activation of G-protein, Src-kinase and also NFkB - signaling pathway. COX-2 is the inducible enzyme that catalyzes the conversion of arachidonic acid to prostaglandin H2 (PGH2, a precursor to other prostaglandins, prostacyclin and thromboxane A2) and activated under certain disorders in the body, including inflammation and carcinogenesis (Seo et al., 2011; Kamath et al., 2001).

The process of free radical oxidation is a component of the metabolic activity of cells that are constantly occurring in living organisms. The main mechanism of activation of free radical processes is to increase the formation of reactive oxygen species (ROS).

Their damaging effect in many cases due to subsequent stimulation of free radical oxidation that leads to oxidative stress (OS), which is manifested by the accumulation of toxic products that damage molecules (including activation of lipid peroxidation and intensification of protein oxidative modification (POM)), membranes of cells, tissues, organs and body in general. The limitation of the negative effects of free radicals is provided by activation of antioxidant system, including through free SH-groups of biothiols and metallothioneins (MT), which exhibit high antioxidant activity in neutralizing ROS and block further oxidative cell damage (Dvorschchenko et al., 2013, Włodek, 2001). Prevalence of free-radical processes of antioxidant defense system is an important pathogenetic link in the origin and development of various diseases.

Because ROS are important for transcription of Ptgs2 (Seo et al., 2011), the relationship between OS, antioxidant element and PAR2-mediated signaling pathways may take place under conditions of dysbiotic changes due to gastric hypochlorhydria.

Probiotic preparations, which play an important role in maintaining the overall homeostasis of the body by optimizing its microecological, are used for the correction of the structural and functional abnormalities in GIT (Culligan et al., 2009). Multi-strain probiotic preparations are more effective compared with monocomponent drugs, because they composed of a combination of different bacterial strains.

For our research we have chosen multi-probiotic Symbiter, which is a symbiosis of living cells biomass of 14 microorganism strains, which are physiological for mammalian intestinal and belong to 10 species: Bifidobacterium bifidum, B. longum, Lactobacillus acidophilus, L. delbrueckii, L. helveticus, Propionibacterium freudenreichii, P. acidipropionici, Lactococcus lactis, Acetobacter aceti, Streptococcus salivarius (Culligan et al., 2009, Iankovsky et al., 2008).

Considering the above, the aim of this research was to assess the intensity of free radical processes and their impact on Par2, Ptgs2 genes expression in rat duodenal epithelial cells under conditions of prolonged gastric hypochlorhydria and with administration of multi-probiotic.

MATERIALS AND METHODS

Animal model

All experiments were carried out on white non-strain male rats with initial weight 180-200 g. Animals were divided randomly into four groups with 8 rats in each of them. Animals treated with 0.2 ml of normal saline i.p. and 0.5 ml of water per os were used as a control (first group). Rats of second group were treated only with multi-strain probiotic preparation “Symbiter® acidophilus concentrated” (manufactured by LLC “O.D. Proslisk”, Ukraine; also referred to as Symbiter) orally in a dose of 140 mg/kg (1014 colony-forming unit per kg) during 28 days in order to estimate the influence of heterogeneous normal microbiota on healthy animals. Hypochlorhydria was evoked through intraperitoneal injection of omeprazole (14 mg per kg of weight once a day) during 28 days (Tsyrriuk et al., 2007); this group represented model of hypochlorhydria associated with dysbiosis (third group). Animals of fourth group were simultaneously treated with omeprazole and Symbiter per os in the same dosage.

Isolation of biological material

After 28 days since the start of experimental animals were sacrificed via decapitation according to “AVMA Guidelines for the Eutanasia of Animals” (2013). The crypts and the villi of duodenal epithelial cells were extracted by the means of a rapid total, protein-bound and non-protein-bound SH-groups content
was conducted by the method of Elman (Ellman, 1959). The relative content of MT was evaluated in partially purified fractions of metalloproteins that received by chloroform - ethanol fractionation of villus and crypt epithelial cells in acidic conditions (Linde et al., 2006). Protein content was determined by the method of Lowry (Lowry et al., 1951). All indexes are listed at 1 mg of protein.

**Semiquantitative RT-PCR**

RNA was isolated following Chomczynski and Sacchi (Chomczynski et al., 1987); cDNA was synthesized in 20 μl of reaction mix containing 2 μg of RNA, 1 mM dNTP, 200 U of reverse transcriptase “RevertAid M-MLV”, corresponding buffer, 20 U of ribonuclease inhibitor “Thermo Scientific RiboLock RNase Inhibitor” (“Thermo Scientific”, Lithuania), 20 pmol of reverse primer.

Synthesis was carried out in the following conditions: 65°C – 5 min, further 45°C – 1 h. PCR was performed in 30 μl of reaction mix containing 3 μl of cDNA, PCR buffer, 200 μM of each dNTP, 30 pmol of each primer, 2.5 mM MgCl₂ and 1 U of Taq DNA polymerase (“Thermo Scientific”, Lithuania). PCR amplifications consisted of the initial denaturing step of 95°C for 3 min, followed by 30 cycles (28 cycles for Actb – gene used as the internal control of reaction due to its constitutive expression) of 95°C for 45 s, appropriate annealing temperature: 53°C – 357 b.p., 49°C – 40 s for Par2 (521 b.p.), the extending step at 72°C for 1 min. And the final extension step was performed at 72°C for 5 min. The following primers were used in reactions (designed using Primer BLAST) – for Par2: forward – GAAATGCACCGGGACCCAA, reverse – TCCCCTATAGTTCCAGTCGT; for Ptg2: forward – TGCTGGTCTCAAAAATGCATGTA, reverse – TGTGCAGAACTCGAGGCTAGT; for Actb: forward – TGAGCAAGATGATGAGAAT, reverse – ATTTGCAGATGTGATGACGT. Separation of PCR products was carried out electrophoretically in 1.6% agarose gel with 0.5x TBE buffer (Green et al., 2012). For semi-quantitative analysis of amplicons expression based on densitometry the Image J 1.48d program (“NIH, USA”) was used. The indices of mRNA expression were calculated in relation to Actb for each sample following Konturek et al. (Konturek et al., 1998).

**Ethics**

Positive findings of ethical expertise were adopted on session of Bioethical Commission of ESC “Institute of Biology” of Taras Shevchenko National University of Kyiv on 26 June 2013. All animals were handled humanely according to rules outlined in “Guide for the Care and Use of Laboratory Animals” (2011), and Order of Ukraine №3447-IV “About defense of animals from abusive handling” from 21 February 2006.

**Statistics**

Statistical processing of experimental results was carried out in “GraphPad Prism 5” (“GraphPad Software Inc.”, USA).

Type of data distribution in groups was checked with Shapiro-Wilk test. As data were distributed normally (p > 0.05), two-way ANOVA was conducted to determine the significance of difference between means with Bonferroni post test. Difference between means was judged as statistically significant if p ≤ 0.05. Mean (M) and standard deviation (SD) were calculated for each group. Pearson’s linear correlation coefficient (r) was used to assess the relationship between specific series. Correlation was estimated as strong for |r| > 0.5, moderate – for 0.3 < |r| ≤ 0.5, and small – for 0.1 < |r| ≤ 0.3.

**RESULTS**

**Content of protein oxidative modification products.**

In animals subjected to prolonged inhibition of hydrochloric acid secretion in stomach the content of POM neutral products with absorption peak of 356 and 370 nm increased: in villus epitheliocytes - 1.7 (p ≤ 0.0001) and 1.8 (p ≤ 0.0001) times; in crypts - 2.1 (p ≤ 0.0001) times compared to the control (Table 1a).

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Analyzed parameter</th>
<th>POM basic products, absorption peak - 430 nm</th>
<th>POM basic products, absorption peak - 530 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control villi</td>
<td>0.072 ± 0.0071*</td>
<td>0.057 ± 0.0053***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.111 ± 0.0092**</td>
<td>0.080 ± 0.0071***</td>
<td></td>
</tr>
<tr>
<td>Symbiter villi</td>
<td>0.062 ± 0.0052**</td>
<td>0.045 ± 0.0042**</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.095 ± 0.0093***</td>
<td>0.063 ± 0.0063***</td>
<td></td>
</tr>
<tr>
<td>Omeprazole villi</td>
<td>0.138 ± 0.0124**</td>
<td>0.107 ± 0.0093***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.185 ± 0.0182****</td>
<td>0.151 ± 0.0131****</td>
<td></td>
</tr>
<tr>
<td>Omeprazole + Symbiter villi</td>
<td>0.061 ± 0.0063***</td>
<td>0.041 ± 0.0044***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.144 ± 0.0151****</td>
<td>0.053 ± 0.0052****</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: *, **, **** – p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 relative to the control; ##, #### – p ≤ 0.01, p ≤ 0.001 relatively animals which only omeprazole treating; ++, ++++, ++++++ - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 villi compared with crypts.

**Table 1b: Content of protein oxidative modification basic products in duodenal epithelial cells of rats under conditions of prolonged hypochlorhydria with the multi-probiotic administration, CU / mg of protein (M ± SD, n = 8).**

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Analyzed parameter</th>
<th>POM basic products, absorption peak - 430 nm</th>
<th>POM basic products, absorption peak - 530 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control villi</td>
<td>0.072 ± 0.0071*</td>
<td>0.057 ± 0.0053***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.111 ± 0.0092**</td>
<td>0.080 ± 0.0071***</td>
<td></td>
</tr>
<tr>
<td>Symbiter villi</td>
<td>0.062 ± 0.0052**</td>
<td>0.045 ± 0.0042**</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.095 ± 0.0093***</td>
<td>0.063 ± 0.0063***</td>
<td></td>
</tr>
<tr>
<td>Omeprazole villi</td>
<td>0.138 ± 0.0124**</td>
<td>0.107 ± 0.0093***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.185 ± 0.0182****</td>
<td>0.151 ± 0.0131****</td>
<td></td>
</tr>
<tr>
<td>Omeprazole + Symbiter villi</td>
<td>0.061 ± 0.0063***</td>
<td>0.041 ± 0.0044***</td>
<td></td>
</tr>
<tr>
<td>crypts</td>
<td>0.144 ± 0.0151****</td>
<td>0.053 ± 0.0052****</td>
<td></td>
</tr>
</tbody>
</table>

Remarks: *, **, **** – p ≤ 0.05, p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 relative to the control; ##, #### – p ≤ 0.01, p ≤ 0.001 relatively animals which only omeprazole treating; ++, ++++, ++++++ - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 villi compared with crypts.
Table 2: Content of SH-groups in duodenal epithelial cells of rats under conditions of prolonged hypochlorhydria and with the multi-probiotic administration, mmol / mg of protein (M ± SD, n = 8)

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Analyzed parameter</th>
<th>Protein-bound</th>
<th>Non-protein</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>villi</td>
<td>2.28 ± 0.191**</td>
<td>0.77 ± 0.071****</td>
<td>2.95 ± 0.283***</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>1.08 ± 0.092***</td>
<td>1.13 ± 0.112****</td>
<td>2.21 ± 0.221***</td>
</tr>
<tr>
<td>Symbiter</td>
<td>villi</td>
<td>2.05 ± 0.182****</td>
<td>0.69 ± 0.062****</td>
<td>2.74 ± 0.242**</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>1.41 ± 0.133****</td>
<td>0.88 ± 0.073****</td>
<td>2.29 ± 0.213**</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>villi</td>
<td>1.29 ± 0.124****</td>
<td>0.61 ± 0.054****</td>
<td>1.92 ± 0.092****</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>0.81 ± 0.072****</td>
<td>0.44 ± 0.042****</td>
<td>1.25 ± 0.121****</td>
</tr>
<tr>
<td>Omeprazole +</td>
<td>villi</td>
<td>1.84 ± 0.143****</td>
<td>0.77 ± 0.063****</td>
<td>2.61 ± 0.214****</td>
</tr>
<tr>
<td>Symbiter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Remarks: ***, **** - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 relative to the control; **, ###, #### - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 relatively animals which only omeprazole treating; --, ++, +++ - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 villi compared with crypts.

In animals upon joint administration of omeprazole and Symbiter the content of POM neutral products with absorption peak of 356 and 370 nm decreased in villi: 2.4 (p ≤ 0.0001) and 2.5 times (p ≤ 0.0001) compared to the third experimental group; and in crypts 1.4 times (p ≤ 0.001) in comparison to the control one. In crypts these indexes were lower: 2.4 (p ≤ 0.0001) and 2.8 (p ≤ 0.0001) compared to the third group, meanwhile the content of POM neutral products with absorption peak of 370 reduced 1.3 times (p ≤ 0.01) in comparison to the control animals.

In rats treated only with Symbiter the content of POM neutral products with absorption peak of 356 and 370 nm decreased: in villi: 1.1 (p ≤ 0.05) and 1.2 (p ≤ 0.01) times respectively, in crypts - 1.4 (p ≤ 0.001) and 1.3 (p ≤ 0.01) times, compared to the control group.

In animals treated with omeprazole the content of POM basic products with absorption peak of 430 and 530 nm increased: in villi - 1.9 (p ≤ 0.0001) and 1.8 (p ≤ 0.0001) times; in crypts - 1.9 (p ≤ 0.0001) times compared to the control (Table 1b).

These parameters in villi of rats from the third group were lower: 2.3 (p ≤ 0.0001) and 2.6 times (p ≤ 0.0001) compared to the third group; in the same time the content of POM neutral products with absorption peak of 530 decreased 1.4 times (p ≤ 0.001) in comparison to the control animals. In crypts cells the content of POM basic products with absorption peak of 430 nm decreased 1.3 (p ≤ 0.0001) times compared to the third group and was 1.3 times higher than the same parameter in the control group. The content of POM basic products with absorption peak of 530 nm decreased 2.8 times (p ≤ 0.0001) compared to the third group and 1.5 times (p ≤ 0.0001) to the first group. In rats treated only with Symbiter the content of POM basic products with absorption peak of 430 and 530 nm was lower: in villi: 1.2 (p ≤ 0.05) and 1.3 (p ≤ 0.01) times respectively, in crypts - 1.4 (p ≤ 0.001) and 1.3 (p ≤ 0.01) times, compared to the control group.

Content of total, protein-bound and non-protein-bound SH-groups

It was found that the content of total, protein-bound and non-protein SH-groups of rats with prolonged inhibition of gastric acid-function decreased: in the epithelium villi - 1.5 (p ≤ 0.0001), 1.8 (p ≤ 0.0001) and 1.3 (p ≤ 0.01) times, respectively, in the crypt - 1.8 (p ≤ 0.0001), 1.3 (p ≤ 0.001) and 2.6 (p ≤ 0.0001) times, accordingly, compared with control (Table 2).

Animals of the fourth group had higher content of total, protein-bound and non-protein SH-groups; in villi: - 1.2 (p ≤ 0.01), 1.4 (p ≤ 0.0001) and 1.3 (p ≤ 0.001) times in accordance, in crypts - 2.6 (p ≤ 0.0001), 2.1 (p ≤ 0.0001) and 3.6 (p ≤ 0.0001) times respectively, in relation to the control group. Besides this, the above mentioned parameters were 1.2 (p ≤ 0.01) lower in comparison to the control indexes in villi, meanwhile 1.5 (p ≤ 0.0001), 1.6 (p ≤ 0.0001) and 1.4 (p ≤ 0.001) times higher accordingly, compared with control. The content of protein-bound and non-protein SH-groups in crypts of animals treated only with Symbiter was 1.3 times (p ≤ 0.001) higher and 1.3 times (p ≤ 0.01) lower in relation to the control.

Metallothioneins content

In animals injected with omeprazole during 28 days the total MT concentration was: in villi - 2 times (p ≤ 0.0001) higher, in crypts - 1.7 times (p ≤ 0.0001) lower relatively to control (Table 3).

Table 3: Content of metallothioneins in duodenal epithelial cells of rats under conditions of prolonged hypochlorhydria and with the multi-probiotic administration, (M ± SD, n = 8).

<table>
<thead>
<tr>
<th>Group of animals</th>
<th>Epithelial cells</th>
<th>µmol / mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>villi</td>
<td>0.198 ± 0.0183****</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>0.285 ± 0.0261****</td>
</tr>
<tr>
<td>Symbiter</td>
<td>villi</td>
<td>0.159 ± 0.0102*****</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>0.491 ± 0.0443*****</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>villi</td>
<td>0.391 ± 0.0353*****</td>
</tr>
<tr>
<td></td>
<td>crypts</td>
<td>0.171 ± 0.0151*****</td>
</tr>
<tr>
<td>Omeprazole +</td>
<td>villi</td>
<td>0.210 ± 0.0194*****</td>
</tr>
<tr>
<td>Symbiter</td>
<td>crypts</td>
<td>0.440 ± 0.0412*****</td>
</tr>
</tbody>
</table>

Remarks: ***, **** - p ≤ 0.01, p ≤ 0.001, p ≤ 0.0001 relative to the control. ### - p ≤ 0.0001 relatively animals which only omeprazole treating; ++++ - p ≤ 0.0001 villi compared with crypts.

In villi of animals upon joint administration of omeprazole and Symbiter the total MT concentration decreased 1.9 times (p ≤ 0.0001) in comparison with the third group. In similar terms, this index in crypts increased: 2.6 times (p ≤ 0.0001) in relation to the third group of animals and 1.5 times (p ≤ 0.0001) relative to controls. MT content in rats injected only with Symbiter
was 1.3 times (p ≤ 0.01) lower in villi and 1.7 times (p ≤ 0.0001) higher in comparison with the control.

A

B

Fig. 1: Level of Par2 gene mRNA in rat duodenum upon long-term hypoacidity and with the multi-probiotic administration. A – RT-PCR electrophoregram, B – relative expression histogram, M – molecular mass marker – “GeneRuler™ 100bp DNA Ladder” (“Fermentas”, Lithuania); villus epithelial cells: 1 – control; 2 – Symbiter; 3 – omeprazole; 4 – omeprazole + Symbiter; crypt epithelial cells: 5 – control; 6 – Symbiter; 7 – omeprazole; 8 – omeprazole + Symbiter; N-PCR – negative PCR control; **** - p ≤ 0.0001 in relation to control; #### - p ≤ 0.0001 compared to the animals treated with omeprazole; ++++ - p ≤ 0.0001 villi in comparison with crypts.

Relative expression level of Par2 gene

The level of Par2 gene expression was 2 times (p ≤ 0.0001) higher of the control value in villi and 7.9 (p ≤ 0.0001) times higher in crypts of the animals treated only with omeprazole for 28 days (Figure 1, A,B). Upon simultaneous administration of the multi-probiotic this parameter was 1.6 times lower in villi compared to the third experimental group (p ≤ 0.0001).

Upon simultaneous administration of the multi-probiotic this parameter was 1.9 times (p ≤ 0.0001) lower in villi compared to the third experimental group. In crypts this index was 2.1 times lower than in the third group of animals (p ≤ 0.0001) and remained 3.4 times (p ≤ 0.0001) higher of the control value. In rats treated only with Symbiter there was no change of this value both in villus and crypt epithelial cells of duodenum in comparison to the control one.

Relative expression level of Ptgs2 gene

We found, that the level of Ptgs2 gene expression was 1.8 times (p ≤ 0.0001) higher compared to the control values in villi and 4.3 times (p ≤ 0.0001) higher in crypts upon long-term hypoacidity (Figure 2, A,B.)

In case of the simultaneous administration of the multi-probiotic the above mentioned parameter was 1.5 (p ≤ 0.0001) and 2.3 times (p ≤ 0.0001) lower in villi and crypts respectively than in the third group, but remained 1.9 times (p ≤ 0.0001) higher of the control value in crypts. In rats treated only with Symbiter there was no change of this value both in villus and crypt epithelial cells of duodenum in comparison to the control one.

Also we found a strong positive correlation between Par2 and Ptgs2 gene expression in villi (r = 0.9, p = 0.021) and crypt epithelial cells (r = 0.9, p = 0.015) under long-term gastric hypochlorhydra and with administration of multi-probiotic.

DISCUSSION

Obtained increase of POM neutral and basis products content is served as a conclusive evidence of intensification of free radical processes in rat duodenal epithelial cells under long-term gastric hypochlorhydra. Recent study showed, that intensification of POM was involved in protein misfolding, the loss of enzymatic activity, violation of membrane reception and assembly of protein complexes (Gamou et al., 1995). Thus, under these conditions is a
massive oxidation of proteins in duodenal villi and crypts with further generation of POM that have destructive properties and cause the development of OS.

In the body free SH-groups are mainly composed of cysteine residues of low molecular weight peptides (non-protein-bound SH-groups) or high molecular weight peptides (protein-bound SH-groups) and show high sensitivity to free radical oxidation. So, OS is correlated with reduced levels of free SH-groups. In current investigation we showed reduction of protein-bound SH-groups content under conditions of prolonged gastric hypoacidity, which may correlate with protein malfunction, loss of their function or degradation in the duodenal epithelial cells (Włodek, 2002).

Decrease of non-protein-bound SH-groups under similar conditions indicated intensification of prooxidant component in duodenal villi and crypts, because the majority of these sulfhydryl groups were the part of the low molecular weight thiol - glutathione, which exhibits antioxidant properties (Meyer et al., 2005). A large number of protein SH-groups are accounted for metallothioneins, which are characterized by low molecular weight, ability to bind heavy metals and antioxidant effects on cells (Linde et al., 2006).

Obtained increase of MT content in duodenal villus epithelial cells under conditions of long hypochlorhydria may be due to the presence of specific sequences in the genes of their promoter, which are capable of binding stress transcription factors - Sp1, AP-1, AP-2 (Davis et al., 2000). In turn, MT reduction shown in crypts under these conditions reflects both the reduction of protein-bound SH-groups (which correlates with our data obtained in the analysis of the content of protein-bound SH-groups) and the weakening of antioxidant protection and development of OS.

The increase of Par2 gene mRNA level under prolonged gastric hypochlorhydria in duodenal villi and crypts may indicate premature activation of proteolytic enzymes, including trypsin, which is the dominant activator of PAR2 (Kawabata et al., 2001). Thus, the obtained elevation of Par2 expression level was consistent with our previous data (Vakal et al., 2015), where we demonstrated the growth of trypsin activity in rat pancreas homogenate under prolonged inhibition of gastric acid-function, since trypsin is one of the major natural activators of PAR2 along with mast cells tryptases.

Moreover, the found presence of pancreatic proteases activated cationic isoforms in pancreatic juice may also be due to enhanced Par2 gene expression, as PAR2 activation promotes secretion of pancreatic juice with dissolved hydrolases in it (Vakal et al., 2015). In addition, activation of PAR2 can cause increased secretion of pancreatic juice with dissolved hydrolases from the duct to the duodenum. In addition, elevated levels of Par2 expression under prolonged gastric hypochlorhydria in duodenal epitheliocytes may indicate the development of inflammation in the background of dysbiotic changes; because was shown, that PAR2 was also activated by mast cells tryptases (Bueno, 2008). Because, the impact of cellular or secretory components of pathogenic microorganisms and (or) load by proinflammatory molecules from endogenous sources of inflammation near duodenum lead to rapid development of inflammation in the organ. This is supported by intensified migration of leukocytes into tissue, followed by growth of proinflammatory cytokines content (TNF-α, IL-1, IL-8 and platelet-activating factor (PAF)), synthesis of cell adhesion molecules, generation ROS and development of OS in duodenum (Seo et al., 2011, McClave, 2012). Recent studies demonstrate that Par2 enhances Ptgs2 expression by means of G-protein activation, Src-kinase in different disorders, for example, in H. pylori-associated gastric carcinogenesis and inflammation. Thus, we found a strong positive correlation between the levels of these genes expression in conditions of prolonged gastric hypoacidity and with the multi-probiotic administration. G_i protein is known to mediate the activation of NF-kB in carcinogenesis and the connection of Src kinase to NF-κB has been reported in toll-like receptor-activated signaling and epidermal growth factor-induced cell proliferation (Seo et al., 2011).

Since NFκB binding site is located in the promoter region of Ptgs2, G-protein and Src-kinase may be associated with activation of this gene expression by NF-κB - signaling pathway in addition to Par2 - mediated signaling pathway. As Ptgs2 gene is responsible for many mitogenic and inflammatory stimuli and ROS are important for transcription of Ptgs2, obtained Ptgs2 upregulation is accompanied by COX-2 and prostaglandin E2 production, accelerated cell proliferation and may be linked with further development of the inflammatory process in the background of dysbiotic changes in duodenal cells (Seo et al., 2011, Kamath et al., 2001).

So, the obtained changes in Par2 gene expression in villus and crypt epithelial cells under prolonged gastric hypochlorhydria indicate the development of pathological processes in the duodenal tissue, including inflammation. Different levels of change in analyzed genes expression in villi and crypts are due to structural and functional features of duodenal cells: presence of poorly differentiated clones of proliferating cells as part of crypts cells, which are less resistance to OS compared to differentiated postmitotic cells. It was also observed, that for the first crypt epithelial cells of the small intestine, including the duodenum were damaged in the early stages of inflammatory diseases, due to migration of neutrophils from blood vessels into the lumen of the crypts, leading to the formation of crypt abscesses (Barrett et al., 2006).

Thus, the results of current research may indicate that the increase of Par2 expression leads to activation of Ptgs2 on the background of free radical processes prevalence of the antioxidant defense system in the development of inflammation in the duodenum on account of dysbiosis in conditions of prolonged hypochlorhydria.

After administrating of Symbiter decrease of POM neutral and basis products content, elevation of total, protein- and non-protein-bound SH-group, MT concentration recovery were also observed in current investigation due to the antioxidant and
anti-inflammatory effect of the multi-strain probiotic preparation. Normalization of Par2 and Ptgs2 gene expression using multi-
probiotic preparation, on the one hand, indicates a lack of prematurely activated protease, on the other - can point to the
important role dsbiotic-inflammatory way in the development of damage in duodenum epithelial cells in conditions of prolonged
hypochlorhydria, because we know that PAR2 receptor is also activated by bacterial proteases (Bueno, 2008). Regarding the
possible mechanisms of multi-probiotic Symbiter influence on gene expression in the duodenum, above all, we should note its
ability to eliminate bacterial colonization in the GIT and dysbiosis, which removes from the GIT and associated organs loads from the
pathogenic microflora (Dvorschkenko et al., 2013, Culligan et al., 2009, Iankovsky et al., 2008). Also, the waste products of bacterial
strains that are represented in the multi-strain probiotic preparation Symbiter (vitamins, exopolysaccharides, short chain fatty acids,
immunomodulators and so on) enhance the barrier function of the intestinal wall, anti-bacterial invasion, have antioxidant properties,
etc., so they can restore normal flora of the GIT, inhibit the development OS and reduce the intensity of the inflammatory and
destructive processes in the duodenum (Dvorschkenko et al., 2013, Iankovsky et al., 2008, Tsryiuk et al., 2007, Lutgendorff et al.,
2008). Further study should be performed to elucidate the specific molecular mechanisms of pathogenic organisms influence on the
duodenum.

CONCLUSION
Current study demonstrates, that prolonged experimental inhibition of acid secretion in stomach was accompanied by the
activation of free radical processes (intensification of protein oxidative modification, free SH-groups pool reduction, changes of
metallothioneins concentration) and elevation of Par2 and Ptgs2 genes expression levels in rat duodenal epithelial cells. Reduction of free radical processes intensity; restoration of redox balance and the normalization of Par2, Ptgs2 gene expression pattern in the duodenum upon the simultaneous administration of the multi-
probiotic Symbiter were observed. Our data indicate that Par2 overexpression leads to activation Ptgs2 on the background of free
radical processes over the antioxidant defense system in the development of inflammation in the duodenum through dysbiotic
changes in the conditions of prolonged hypochlorhydria.

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